(54) Title: IMPLANTABLE MATERIALS

(57) Abstract

Polymer hydrogels are adapted for surgical implants by chemical modification of the surface to stimulate the attachment and growth of cells thereto. The modification may be by oxidative acid etching or by copolymerisation with methacrylic acid and diethylaminoethyl methacrylate.

IMPLANTABLE MATERIALS

TECHNICAL FIELD

This invention relates to implantable materials and to processes for their manufacture.

5   ### BACKGROUND ART

Blood vessels and naturally occurring internal organs are lined with a thin layer of endothelial cells which have a number of bio-chemical functions. In so far as surgical implants are concerned, one important function of

10   endothelial cells is their involvement in the processes of rendering the surfaces of blood vessels non-thrombogenic.

A key factor in attaining a non-thrombogenic vascular graft is the rapid development of a lining of the endothelial cells on the implant. Thus, such implants

15   benefit from having surfaces that encourage endothelial cell attachment and spreading.

Similar considerations apply in respect of other implants that are intended for prolonged implantation where blood contact is required such as permanent

20   indwelling catheters for drug administration, fluid drainage tubes, vascular shunts, pacemaker leads and implantable transducers.

Synthetic polymer hydrogels have found a wide range of biomedical applications, including controlled drug

25   delivery systems, replacement blood vessels, would dressings, coatings for biosensors, soft tissue substitution and contact lenses.

As a family of polymeric materials, synthetic hydrogels are generally well tolerated when implanted <u>in</u>

<u>vivo</u> and can be tailored to suit the many potential
functions of prosthetic devices in contact with blood or
soft tissues. The success of hydrogels as biomaterials
lies partially in their superficial resemblance to living
5   tissue, a property attributable to their relatively high
water content (say 20%-99%), which immediately results in
minimal frictional irritation of surrounding tissues.

In addition, hydrogels can be non-toxic, chemically
stable and (due to their water content) can exhibit a low
10   interfacial tension with aqueous environments. This
latter property becomes particularly important in
considering the compatibility of blood-contacting
surfaces, where minimal interfacial tension has been
related to thromboresistance.

15   The hydrogel polyHEMA - poly(2-hydroxyethyl
methacrylate) - is known to possess inherent
characteristics of good permeability, water uptake and
tolerable polymer/tissue interface disruption which make
it a desirable biomaterial (see, for example, Cohn D et al
20   (1984) Radiation - Grafted Polymers for Biomaterial
Applications I. 2-Hydroxyethyl Methacrylate: Ethyl
Methacrylate Grafting on to Low Density Polyethylene Films
<u>J. App. Pol. Sci. 29</u>, 2645-2663).

Despite these advantages, however, unmodified
25   polyHEMA does not have the ability to sustain mammalian
cell growth and consequently its use as a biomaterial has
been restricted to applications where this inability is a
positive advantage (see Andrade J.D. (1975) Hydrogels for
Medical and Related Applications <u>ACS SYMPOSIUM SERIES 31</u>,

Washington).

Recent investigations into the effects of treating polystyrene with sulphuric acid (see Curtis A.S.G. et al (1983) Adhesion of Cells to Polystyrene Surfaces

5   J. Cell Biol 97, 1500-1506) have shown a marked improvement in the ability of that polymer to support mammalian cell growth after acid etching. Whilst this is not a new concept, modern analytical methods such as electron spectroscopy for chemical analysis have allowed a

10   more detailed study of surface changes occurring with such treatments resulting in some clarification of certain aspects of cell adhesion.

Another disadvantage of polyHEMA is that its poor mechanical properties prevent it from being used as an

15   implant requiring high mechanical strength.

By copolymerisation of polyHEMA with other selected synthetic polymers, it has been possible to manipulate surface charges, hydrophilicity and equilibrium water content to achieve varying degrees of attachment and

20   growth of fibroblastoid cells. An alternative modification has been to incorporate natural polymers such as collagen, elastin and fibronectin in polyHEMA hydrogels. This has provided a model system to study the contribution of such extracellular matrix components to

25   cell adhesion and growth. While this approach has allowed the growth of a wider variety of cell types on such hydrogels, it also places other restrictions and problems on the polyHEMA system as a biomaterial for use in prosthetic devices.

It is an object of this invention to provide an implantable material having improved biocompatibility arriving from enhanced endothelial cell attachment properties.

5   It is a further object of this invention to provide an improved implantable material having a mechanically acceptable substrate to which is attached a polyHEMA layer.

DISCLOSURE OF THE INVENTION

10   According to the invention there is provided an implantable material comprising a hydrogel the surface of which is chemically modified so as to stimulate the attachment and growth of cells thereto.

The chemical modification may consist of oxidative
15   etching of the hydrogel or copolymerisation of the hydrogel with methacrylic acid.

The surface of a hydrogel of polyHEMA may also be modified by limited surface hydrolysis.

By exposing polyHEMA to a particular acid treatment
20   we have achieved cell attachment and cell growth rates comparable with those of tissue culture polystyrene and better than P.T.F.E. (TEFLON), a commonly used biochemical.

The treated hydrogel of the invention has a surface
25   that supports efficient adhesion of endothelial cells which grow to confluence. Electron spectroscopy chemical analysis of the acid etched polyHEMA indicates an increase in C=O groups relative to C-O groups suggesting that increased negative charge from carboxyl groups contribute

to the change in cell-substatum interaction. This contention is supported by the fact that methacrylic acid contributes mainly to increased carboxyl groups in the material.

5   The cell plating efficiency of an acid etched polyHEMA treated in accordance with the invention increased from 0% to 95% of that of glow-discharge treated polystyrene and fibronectin binding capacity increased from 0 to 2×10^{-11} pico mole per square centimetre.

10   The invention also provides an implantable material consisting of a mechanically acceptable substrate having a hydrogel layer, said hydrogel layer being chemically modified as described above so as to stimulate the attachment and growth of cells thereto.

15   The substrate may be any convenient material such as polyurethane, TEFLON, DACRON or other plastic material, platinum, titanium or other metal as well as carbon and ceramic materials. The polyHEMA may be attached to the substrate by mechanically keying it to a microporous

20   structure or by grafting in the case of a polymer surface.

According to another aspect of the invention, there is provided a method of providing an implant comprising the steps of:-

    a)   forming a substrate having pre-determined

25   mechanical properties,

    b)   applying a hydrogel layer to the substrate, and,

    c)   chemically modifying the surface of the hydrogel
          so as to stimulate the attachment and growth of
          cells thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the invention may be more readily understood and put into practical effect, reference will now be made to the accompanying drawings in which:-

5   Fig. 1 is a graph of percentage cell attachment as a function of etching time for a polyHEMA substrate etched with sulphuric acid,

     Fig. 2 is a graph of B.A.E. cell growth as a
10   function of time for sulphuric acid etched polyHEMA and glow-discharge treated polystyrene,

     Fig. 3 is a pictorial representation of the protein binding characteristics of etched
15   and non-etched polyHEMA and rough and smooth P.T.F.E., and,

     Fig. 4 is a graph of percentage cell attachment as a function of percentage copolymer in polyHEMA after copolymerising charged HEMA
20   monomer and with methacrylic acid and diethylaminoethyl methacrylate (DEAEMA).

BEST MODE FOR CARRYING OUT THE INVENTION

The invention will now be described in more detail with reference to the following examples.

25 EXAMPLE 1: MATERIALS

The material utilised in the examples were:-

(i) hydroxyethyl methacrylate (HEMA), Methacrylic Acid (MAA), Diethyl aminoethyl methacrylate (DEAEMA) and Tetraethylene glycol dimethacrylate (TECDMA).

(ii) [^{14}C] Methylated Human fibroacctin (specific activity 1.4Ci/mMol) and Bovine Serum Albumin (specific activity 3.5Ci/mMol)

(iii) NCS solubiliser for Liquid scintillation
5 counting.

(iv) Bovine plasma fibronectin (FN) prepared as described in G.N. Hannan, J.W. Redmond, and B.R. McAuslan, "Similarity of the carbohydrate moieties of fibronectins derived from blood plasma and synthesised by cultured
10 endothelial cells," Biochim. Biophys. Acts, 801, 396 402 (1984).

(v) Virgin, unfilled P.T.F.E. (TEFLON, Registered Trade Mark) sheet, 0.25 mm thick

(vi) Segmented polyurethane sheet, 0.2 mm thick was
15 cast from commercially available "Blumer" solution.

Preparation of polymers

PolyHEMA homopolymer was prepared essentially as described by Civerchia-Perez et. al., (L. Civerchia-Perez, B. Faris, G. LaPointe, J. Beldekas, H. Leibowitz, and C.
20 Franzblau, "Use of collagen-hydroxyethylmethacrylate hydrogels for cell growth," Proc. Natl. Acad. Sci. USA, 77(4), 2064-2068 (1980)) except that the casting of the hydrogels was carried out in a Bio-Rad Slab-gel pouring apparatus using 0.75 mm thick Teflon spacers. By varying
25 the thickness of the Teflon spacers gels were successfully cast from 0.25 mm to 1 mm in thickness.

The copolymer hydrogels of polyHEMA/MAA and polyHEMA/DEAEMA were prepared as described by Holly and Refojo (F.J. Holly, and M.F. Refojo, "Wettability of

hydrogels I. Poly (2-hydroxyethyl methacrylate),"

J. Biomed. mater. Res., 9, 315-326 (1975)), again casting

was done in the Bio-Rad apparatus.

After polymerisation, the hydrogels were cut into 14

mm diameter discs and dialysed extensively against

phosphate buffered saline pH 7.4.(PBS) It was found that

dialysing the membranes prior to cutting into discs was

not as efficient with the larger sizes that were able to

be cast in the Bio-Rad system. Some swelling of the discs

did occur as a result of dialysis but they fitted

readily into a 16 mm diameter tissue culture well.

After dialysis the buttons were transferred to PBS

containing penicillin, streptomycin and kanamycin and

stored at 4°C. Sterilisation by UV for 2 hours was

carried out immediately prior to use for cell studies and

the sterilised discs equilibriated with an appropriate

sterile cell culture medium for one hour. The teflon

sheet was cut into 15 mm diameter discs and the

polyurethane sheet was cut into squares of 1 cm x 1 cm.

Both materials were washed extensively with acetone and

then absolute ethanol. Sterilisation was achieved by

prolonged soaking in absolute ethanol with washes in

sterile PBS, or by autoclaving. No detectable differences

were obvious from using either method before cell or

protein binding assays.

Surface modification of polyHEMA

PolyHEMA homopolymer discs were treated individually

as follows.

A disc was placed into a specially constructed

polyallomer ladle (2.5 cm in diameter, 2 cm deep with a
6.5 cm x 0.75 cm handle, 3 cm diameter perforations were
punched out of the bottom of the ladle with a cork borer)
and quickly immersed into acid (either sulphuric 98%,

5 hydrochloric, consisting of adding 3 vol. 70% perchloric
acid and 2 vol. of saturated aqueous potassium chlorate or
hydrofluoric 50%) for predetermined times ranging from 1
to 20 seconds. The perforations in the base of the ladle
allowed the acid to act equally on all hydrogel surfaces

10 by creating a turbulent upwelling of acid that suspended
the disc in the centre of the ladle. Gentle vertical
agitation of the ladle maintained this action. After acid
immersion, the ladle and disc were immediately washed in
deionised water several times and the disc then

15 transferred to PBS for extensive dialysing. The discs
turned opaque in a fashion similar to those that were
first dialysed after the initial casting of the hydrogel,
but quickly cleared. After dialysis the discs were stored
and subsequently used in the same manner as described in

20 the hydrogel preparation.

Basic hydrolysis of polyHEMA was carried out at both
room temperature and at 78°C by immersing discs into
solutions of 3.0 M NaOH for times ranging from 30 minutes
to 6 hours. Subsequent washing and dialysis of treated

25 discs was the same as described for the acid treated
samples.

Cell culture and cell growth rate determination

A clonal line of normal bovine aortal endothelial
cells (BAE) was grown and maintained as previously

described.

Single hydrogel discs, Teflon discs and polyurethane squares were placed into separate wells of a Costar cluster dish (24 wells, 16 mm diameter). 1 ml suspensions

5   of 5×10^4 cells were added to each well and routinely maintained in medium 199 plus 10% fetal calf serum.

To determine cell numbers discs were transferred from wells to 35 mm diameter polystyrene tissue-culture dishes and fixed with 2.5% glutaraldehyde. Counts were obtained

10   for a superimposed grid area representing 0.106 mm^2 by using a Bioquant Image Analysis system coupled with an Olympus BH-2 phase contrast microscope. Cell numbers are given as the average and standard deviation of 15 random counts on each of 4 discs, and are expressed per cm^2.

15   For translucent materials such as Teflon and polyurethane the cells were stained with Giemsa prior to counting.

Cell attachment determination

Cells were plated onto the various samples as

20   described above and incubated for 6 hrs at 37°C. After incubation the cells were washed with sterile PBS to remove those not attached. Cells on the translucent samples were fixed and stained as described, then all samples counted on the Bioquant System. Cell numbers are

25   given as an average and standard deviation of 15 random counts on each of 4 replicates per sample and expressed as a percentage of equivalent cells attached per cm^2 to glow-discharge polystyrene.

EXAMPLE 2:   CELL RESPONSE TO ACID ETCHED POLYHEMA

Bovine Aortal Endothelian (B.A.E.) cells were plated onto a series of polyHEMA substrates that had been exposed to the following sulphuric acid etching treatment times.

5

     (a)   nil

     (b)   1 second

     (c)   2 seconds

     (d)   5 seconds

     (e)   10 seconds

10

     (f)   15 seconds

The percent cell attachment as a function of the sulphuric acid etching time for the polyHEMA substrates is shown in Fig. 1. The percent cell attachment for hydrochloric acid and hydrofluoric acid etched polyHEMA

15

substrates is also shown in Fig. 1 to emphasise the efficacy of the sulphuric acid etch.

EXAMPLE 3:   RELATIVE PLATING EFFICIENCIES OF B.A.E.

                CELLS ON ETCHED POLYHEMA v. TEFLON

Bovine Aortal Endothelial (B.A.E.) cells were plated

20

onto a sulphuric acid etched polyHEMA substrate and onto a P.T.F.E. (TEFLON) substrate and the number of cells attached after six hours was expressed as a percentage of the number that attached to tissue culture polystyrene. The results were:-

25

      TEFLON: 70 - 72%

      Etched PolyHEMA: 90 - 95%

The sulphuric acid etched polyHEMA was relatively more efficient in its plating efficiency with respect to tissue culture polystyrene than the P.T.F.E.

EXAMPLE 4:   COMPARATIVE CELL GROWTH OF B.A.E. ON

ETCHED POLYHEMA AND TISSUE CULTURE POLYSTYRENE

The growth of B.A.E. cells (number per square

millimetre) at 3, 6 and 10 days is shown in Fig. 2 for

5   sulphuric acid etched polyHEMA and glow-discharge treated

polystyrene. B.A.E. cell growth to confluence on etched

polyHEMA was comparable to that on tissue culture

polystyrene both in terms of growth rate and morphology.

EXAMPLE 5:   PLATELET BINDING

10   Human platelets were prepared from fresh blood and

labelled with ^{51}Cr (spec. act. 635 uCi/ml) essentially as

described by Dacie and Lewis (J.V. Dacie, and S.M. Lewis,

Practical Haematology, 5th. edn., Churchill Livingstone,

Edinburgh (1975). Samples of polymers were mixed with

15   labelled platelets (conc. 5.0×10^8/ml) and agitated gently

for 2 hrs. After mixing the polymers were washed three

times with PBS containing 1% BSA then counted. The cell

numbers of platelets remaining bound were expressed per

cm^2 of sample.

20   Because the application of an endothelial cell

binding material to vascular prostheses is ultimately

linked to the problem of thrombogenicity, at least a

preliminary indication of the thrombogenic potential of a

material might be gained from its propensity for platelet

25   binding. From the results obtained (Table III) it is

clear that the affinity of platelets for "etched" pHEMA

was much greater than for segmented or any of the other

polymers tested.

EXAMPLE 6:   PROTEIN BINDING

     Materials to be tested were placed individually into
Costar cluster dish wells.   0.5 ml of sterile PBS was
added to each well, then an appropriate aliquot of either

5   [^{14}C] methylated Human fibronectin or [^{14}C] methylated
bovine serum albumin added to give a final concentration
of 0.1 uCi per well.   The wells were incubated for 45
mins at 37°C.   After incubation the materials were
transferred to 20 ml of fresh PBS and washed for one hour,

10   a further rinse in 5 ml of PBS was carried out prior to
transferring the materials to liquid scintillation vials.
4 ml of NCS tissue-solubilising solution was added to each
vial and then the vials were incubated at 50°C for 2
hours.

15   After cooling to room temperature, an appropriate
scintillant was added and the samples dark equilibriated
at 4°C overnight prior to counting.   The amount of
fibronectin and bovine serum albumin bound is the average
obtained from 3 buttons per sample and expressed as a

20   percentage of that bound per cm^2, to glow-discharge
treated polystyrene tissue culture dishes.

    Initial studies on the propensity of sulphuric acid
treated ("etched") polyHEMA to bind blood proteins were
conducted with bovine serum albumin and fibronectin.   For

25   comparison we used T/C polysty, Teflon and a segmented
polyurethane (Biomer).   The results are presented in Table
II and Fig. 3.   PolyHEMA was remarkable in that it showed
very weak binding of albumin compared with the other
polymers.   Acid "etching" did not change this

quantitatively. It is known that pHEMA does not bind
fibronectin, a major adhesive component of the
extracellular matrix. Although acid "etching" did bring
about a measurable change, the resultant fibronectin
5 binding capacity was of the order of 10 fold less than for
the other polymers studied.

Electron spectroscopy for chemical analysis (ESCA)
was used to determine chemical changes in the polymer
surfaces. Replicate samples of polyHEMA hydrogel treated
10 with either chloric, hydrofluoric or sulphuric acids were
prepared. Samples of each were assayed to ensure their
cell adhesive properties were as expected and parallel
samples were submitted for ESCA analysis.

For each acid used the most obvious alteration to the
15 polyHEMA surface was indicated by spectral shifts. These
changes indicated a significant chemical modification
consistent with the creation of surface -COOH groups.
Problems raised regarding the correlation of apparent
surface changes to biological responses are discussed
20 below.

The results are shown in Fig. 3. PolyHEMA was
remarkable in that it showed very weak binding of albumin
compared with the other polymers. Acid etching did not
change this quantitatively. It is known that polyHEMA
25 does not bind fibronectin, a major adhesive component of
the extracellular matrix. Although acid etching did bring
about a measurable change, the resultant fibronectin
binding capacity was of the order of 10 fold less than for
the other polymers studied.

EXAMPLE 7:　ENDOTHELIAL CELL ATTACHMENT TO COPOLYMER
　　　　　　　　HYDROGELS

　　　　Very little is known about the specific molecular
requirements for endothelial cell adhesion in vascular
5　prostheses.　The polyHEMA hydrogel with its neutrally
charged hydroxyl rich surface is a potentially useful
system to explore the requirements for cell attachment,
but it has been established that mammalian cells will not
adhere to and grow on hydrogels of polyHEMA homopolymer.

10　　　　To follow the effects of introducing charged groups
into the polyHEMA surface a series of hydrogels were
prepared from copolymers of HEMA with increasing amounts
of methacrylic acid (MAA) or HEMA with increasing amounts
of diethylaminoethyl methacrylate (DEAEMA), thus
15　introducing negative carboxyl (-COOH) or positive (amino)
charges respectively.

　　　　Using endothelial cell attachment (and subsequent
growth) as parameters, these copolymers were compared to
glow-discharge treated tissue culture grade polystyrene.
20　The attachment capacity of polystyrene for endothelial
cells was set at 100% for comparison.　When compared with
polystyrene the attachment of vascular endothelial cells
determined as a plating efficiency, was increased from
negligible levels on polyHEMA homopolymer to levels of
25　about 50% after copolymerisation with either MAA or DEAEMA
(Fig. 4).　Optimal levels were 20% DEAEMA or 30% MAA v/v.
Beyond these levels DEAEMA inclusion caused cytotoxic
effects leading to cell detachment and arrest of growth.

　　　　Methacrylic acid inclusion caused no deleterious

effect on cell morphology or growth, but beyond addition
to 30% v/v the hydrogel became friable and so physically
altered as to be impractical to handle.

5   The above results discussed in relation to Examples 2
to 7 suggested that either positive or negative charge
groups could affect endothelial cell adhesion. The
introduction of carboxyl groups was focused on as were
other means of introducing such surface charges. The
change in properties of polystyrene by acid oxidation is
10   well documented and we tried a similar approach with
polyHEMA. PolyHEMA hydrogel buttons were treated with
either sulphuric acid hydrofluoric acid or chloric acid.
The latter has been shown to be highly effective in
creating a cell adhesive surface on polystyrene. The
15   buttons treated for varying times were subsequently washed
free of acid then tested for ability to support
endothelial cell attachment and growth.

Treatment with either chloric or hydrofluoric acids
over a wide range of times caused no demonstrable change
20   in the cell adhesion properties of polyHEMA. In contrast
sulphuric acid treatment profoundly altered the surface of
the hydrogel such that it became excellent for the
adhesion and spreading of vascular endothelial cells (Fig.
6). The morphologic appearance of cells, 24 hours after
25   seeding onto sulphuric acid etched polyHEMA, is shown
(Fig. 7) and, provided the optimal etch time (10 sec) was
not exceeded, was indistinguishable from those grown on
tissue culture grade polystyrene (T/C polysty). The
efficiency of attachment of cells to sulphuric acid

treated polyHEMA was compared also to Teflon and segmented

polyurethane (Table I). The results suggest that etched

polyHEMA·in this respect was practically as effective as

T/C polysty and better than Teflon or polyurethane.

5   EXAMPLE 8:   ENDOTHELIAL CELL ATTACHMENT TO ALKALI-TREATED

POLYHEMA

In order to determine if cell attachment to surface

modified polyHEMA could be induced by non-acid means,

several discs were subjected to base hydrolysis. No

10   obvious physical change to the surface of the discs was

noted in any of the treatments with 3.0 M NaOH at room

temperature. Those discs subjected to increasing times in

3.0 M NaOH at 78°C, however, showed signs of surface

cracking after 6 hours (that was similar to a 15 second .

15   treatment with sulphuric acid). Subsequent testing of all

the alkali treated discs failed to reveal any changes in

the ability of the hydrogel to support cell attachment and

growth.

EXAMPLE 9:   CELL GROWTH RATE ON ACID-TREATED POLYHEMA

20   Sulphuric acid-treated polyHEMA hydrogel was compared

to T/C polystyrene for their ability to support growth of

aortal endothelial cells (BAE). No significant difference

between them was found in respect to rate of cell growth

and final cell density achieved.

25   The failure of polyHEMA to support adherence of

mammalian cells is well documented. A contrary report

often cited in reference to cell growth control has been

dismissed as an artefact of discontinuous cell-substratum

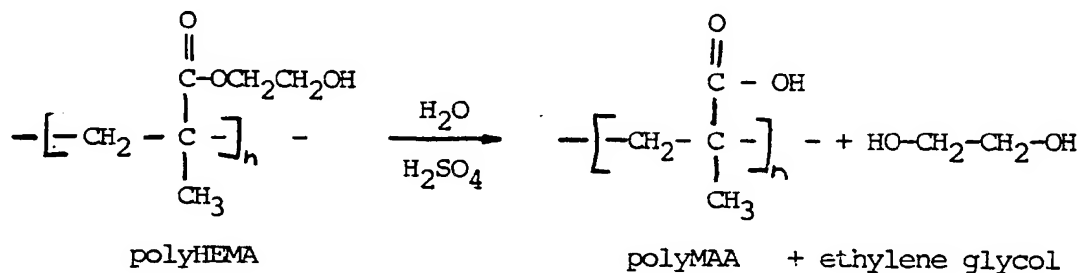interactions. The results show that brief exposure of a

polyHEMA hydrogel surface to concentrated sulphuric acid results in a substratum to which vascular endothelial cells attach and grow virtually as well as they do on the best available tissue culture grade of glow-discharge treated polystyrene. Preliminary results indicate that a wide range of mammalian cells will grow on "etched" polyHEMA. The "etched" polyHEMA surface gave a more uniform attachment and subsequent growth of endothelial cells than did Teflon and this may be advantageous where preliminary endothelial cell seeding can be employed and where uniform surface repopulation is desirable.

Early work on polystyrene "etching" first centred on sulphonic groups for cell attachment. This idea was subsequently discounted and later studies concluded that introduction of -OH groups rather than -COOH groups were essential for mammalian cell attachment. This does not appear likely in the polyHEMA system where it is initially an hydroxyl rich surface that is rendered adhesive following the introduction of -COOH groups.

ESCA analysis of the sulphuric acid treated polyHEMA indicated that the major change was due to creation of -COOH groups which would have resulted in an increase in negative charges on the hydrogel surface. This was confirmed by observations on a corresponding increase in the affinity for cationic dyes (e.g. Crystal Violet, Acridine Orange). This analysis also correlates with the enhanced cell attachment property introduced by copolymerisation with methacrylic acid.

Preliminary evidence suggests that glycol was a by-

product of the "etching" procedure. Thus we propose that
a likely effect of brief sulphuric acid "etching" is to
enhance cell interaction by a limited hydrolysis of
polyHEMA to produce polymethacrylic acid as shown:-

$$-\left[-CH_2-\underset{\underset{CH_3}{|}}{\overset{\overset{\displaystyle C-OCH_2CH_2OH}{|}}{C}}-\right]_h - \quad \xrightarrow[H_2SO_4]{H_2O} \quad -\left[-CH_2-\underset{\underset{CH_3}{|}}{\overset{\overset{\displaystyle C-OH}{|}}{C}}-\right]_h - \; + \; HO-CH_2-CH_2-OH$$

polyHEMA   polyMAA   + ethylene glycol

5   Methacrylic acid groups on the hydrogel surface may also
sufficiently alter the degree of hydration to promote cell
attachment.

     Hydrochloric or hydrofluoric acid "etching" lead to
reformation of surface carboxyl groups and surfaces which

10   presented similar spectra to that elicited by sulphuric
acid "etching". However, hydrochloric or hydrofluoric
treatment consistently failed to change the adhesive
characteristics of polyHEMA for cells. The hydrolytic
treatment of polyHEMA under alkaline conditions, however,

15   demonstrates that simple hydrolysis by acid or basic means
elicits changes that do not necessarily lead to conditions
suitable for cell attachment. Conceivably higher cross-
linking or specific -COOH orientation may occur with those
treatments that fail to produce such surface modification.

20   It is conceivable that attachment of cells to
"etched" polyHEMA is mediated by a serum protein such as
fibronectin. The small but significant increase in

-20-

ability of polyHEMA to bind fibronectin following acid
"etching" is not inconsistent with this idea.   However
hydrochloric acid or hydrofluoric acid "etching" hydrogels
bound fibronectin just as efficiently (Table II) but these

5   did not support cell attachment.   Therefore it is unlikely
that cell attachment to sulphuric "etched" polyHEMA is
simply a result of its ability to bind fibronectin.

Various modifications may be made in details of
composition and of the process without departing from the

10   scope and ambit of the invention.

TABLE I

Polymer	Cell Attachment (6 hr) $\%$ per cm^2*
T/C polysty	100
Etched polyHEMA	90
Teflon	75
Polyurethane	73
polyHEMA	1

* T/C polysty set as maximal attachment.

Relative attachment of BAE cells to various polymers. Numbers attached were expressed as a percentage of the number of cells attaching to T/C polysty.

TABLE II

FN and BSA Binding

	FN		BSA	
	$pmol.cm^2$	%	$pmol.cm^2$	%
polysty	1.31	100	1.20	100
polyHEMA	0	0	0.03	2.5
etched polyHEMA (H_2SO_4)	0.21	16.0	0.03	2.5
etched polyHEMA (Chloric)	0.14	10.7	0.02	1.7
Teflon (P.T.F.E.)	1.74	132.8	1.88	156.7
polyurethane (Biomer)	1.34	102.3	2.03	169.2

Binding of fibronectin (FN) and bovine serum albumin (BSA) to polymers.

TABLE III

Relative Platelet Binding

Polymer	No. Platelets Bound/cm^2
polyHEMA	1.0×10^5
polyHEMA etched	2.0×10^6
P.T.F.E. (Teflon)	6.8×10^5
polyurethane (biomor)	6.5×10^5

Human platelets prepared from fresh blood were labelled with ^{51}Cr. Labelled platelets were incubated with polymer samples and number of platelets bound per cm^2 in 12 hours from the known specific activity of the inoculum and the radioactivity bound to samples.

CLAIMS

1.   An implantable material comprising a hydrogel the
surface of which is chemically modified so as to stimulate
the attachment and growth of cells thereto.

2.   An implantable material comprising a hydrogel
modified by the creation of additional carboxyl groups so
as to stimulate the attachment and growth of cells
thereto.

3.   An implantable material comprising a hydrogel
modified by the creation of amino groups so as to
stimulate the attachment and growth of cells thereto.

4.   An implantable material according to any one of
Claims 1 to 3 wherein the hydrogel is a synthetic
hydrogel.

5.   An implantable material according to any one of the
preceding claims wherein the hydrogel is
poly(2-hydroxyethyl methacrylate).

6.   An implantable material according to any one of the
preceding claims wherein said modification is effected by
etching of the hydrogel by an oxidative acid.

7.   An implantable material according to Claim 6 wherein
said oxidative acid is sulphuric acid.

8.   An implantable material according to Claims 6 or 7
wherein said acid etching is carried out for a period of
less than 15 seconds.

9.   An implantable material according to Claim 5 wherein
the modification comprises copolymerisation of 2-
hydroxyethyl methacrylate monomer with methacrylic acid or
diethylaminoethyl methocrylate.

10. An implantable material according to Claim 9 wherein
the amount of methacrylic acid is from 5 to 40% of the
hydrogel.

11. An implantable material according to Claim 10 wherein
the amount of methacrylic acid is 30% of the hydrogel.

12. An implantable material according to Claim 10 wherein
the amount of diethylaminoethyl methacrylate is 20% of the
hydrogel.

13. An implantable material comprising a base having
thereon a layer of material according to any one of the
preceding claims.

14. A method of forming an implantable material
comprising the steps of:-
     (a)   forming a substrate having pre-determined
          mechanical properties,

(b) applying a hydrogel layer to the substrate, and,

(c) chemically modifying the hydrogel so as to stimulate the attachment and growth of cells thereto.

15. A method according to Claim 14 wherein the modification consists of etching by an oxidative acid.

16. A method according to Claim 15 where said oxidative acid is sulphuric acid.

17. A method according to Claim 15 or 16 wherein said acid etching is performed for a period of less than 15 seconds.

18. A method according to any one of Claims 14 to 17 wherein the hydrogel is poly(2-hydroxyethyl methacrylate).

19. A method according to Claim 18 wherein the modification comprises copolymerisation.

20. A method according to Claim 19 wherein the amount of methacrylic acid is from 5 to 40% of the hydrogel.

21. A method according to Claim 19 wherein the amount of methacrylic acid is 30% of the hydrogel.

22.   A method according to Claim 19 wherein the amount of diethylaminoethyl methacrylate is 20% of the hydrogel.
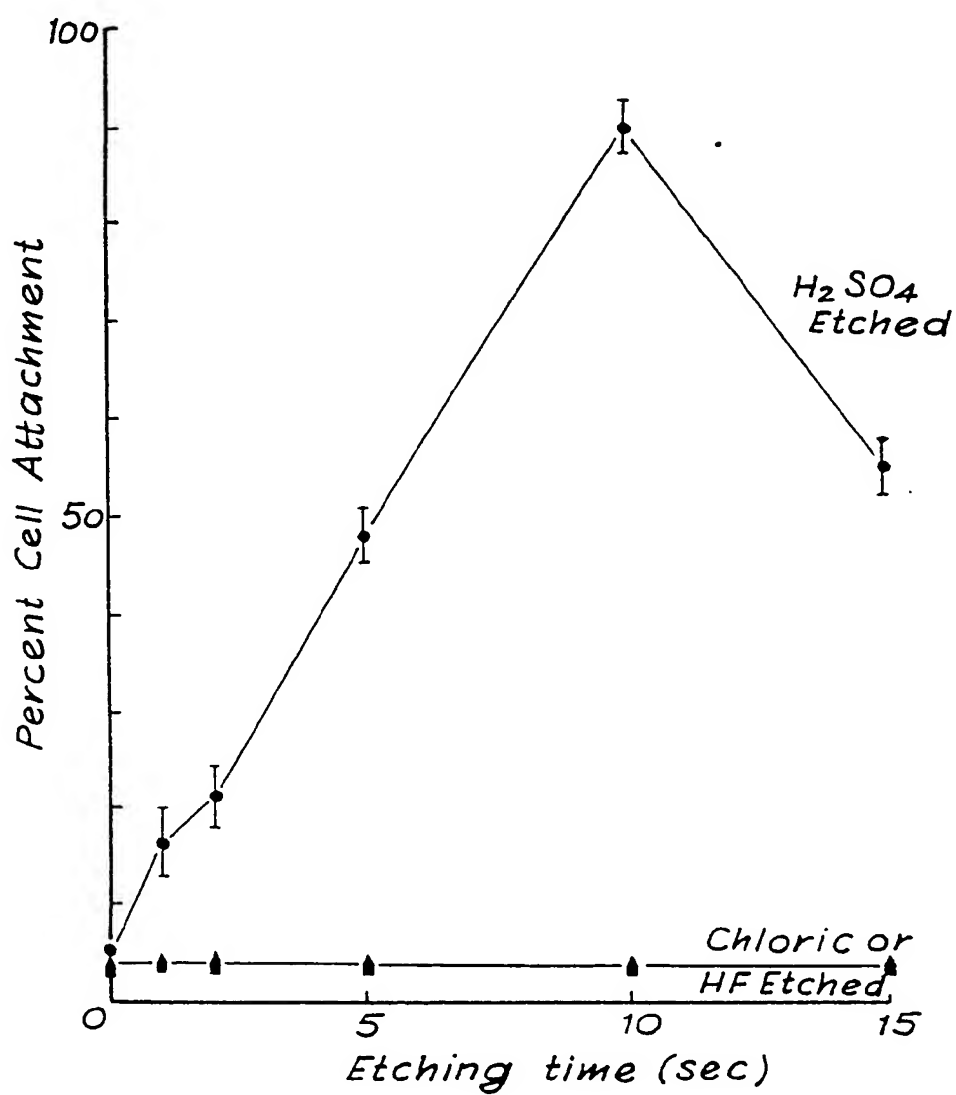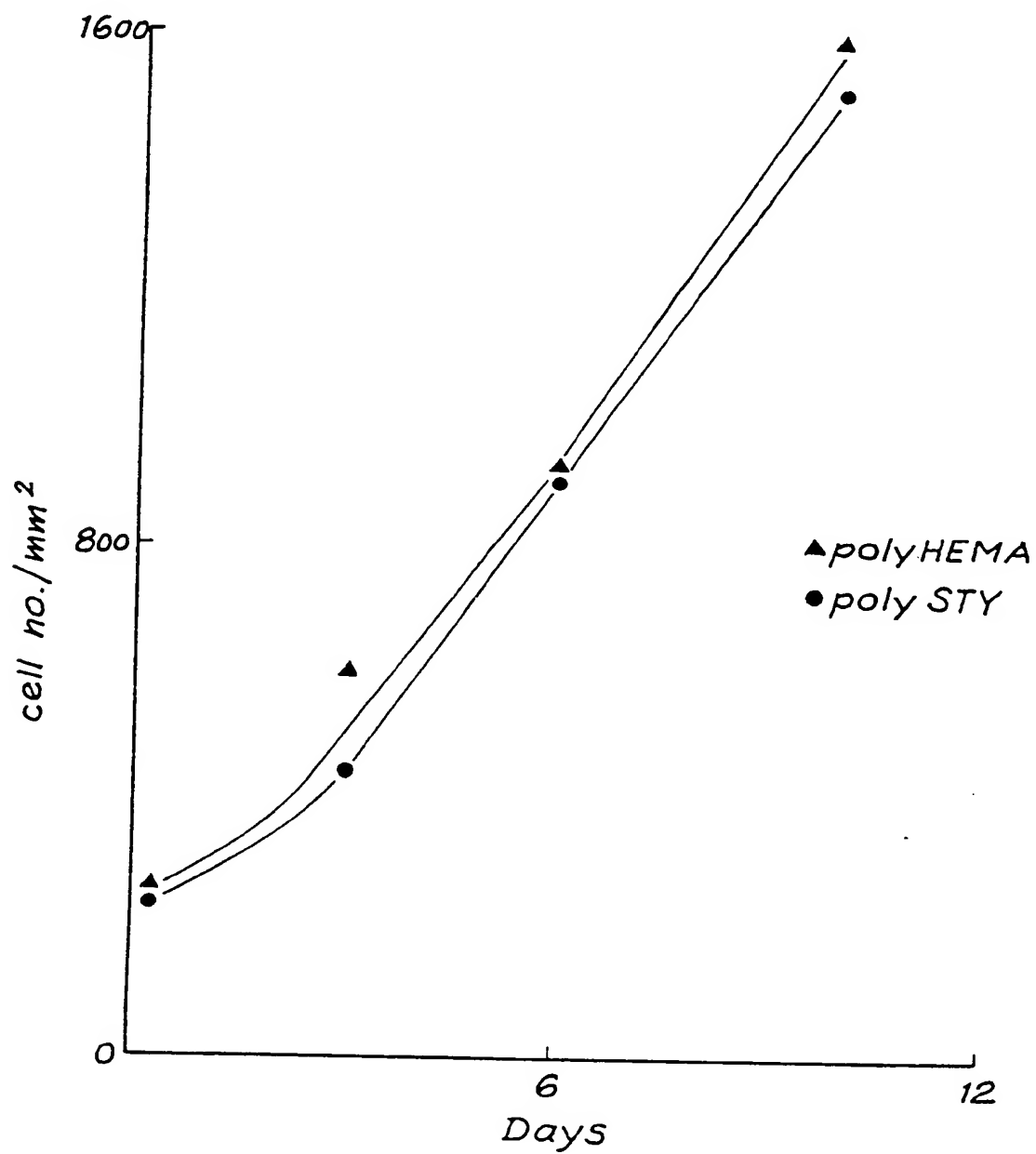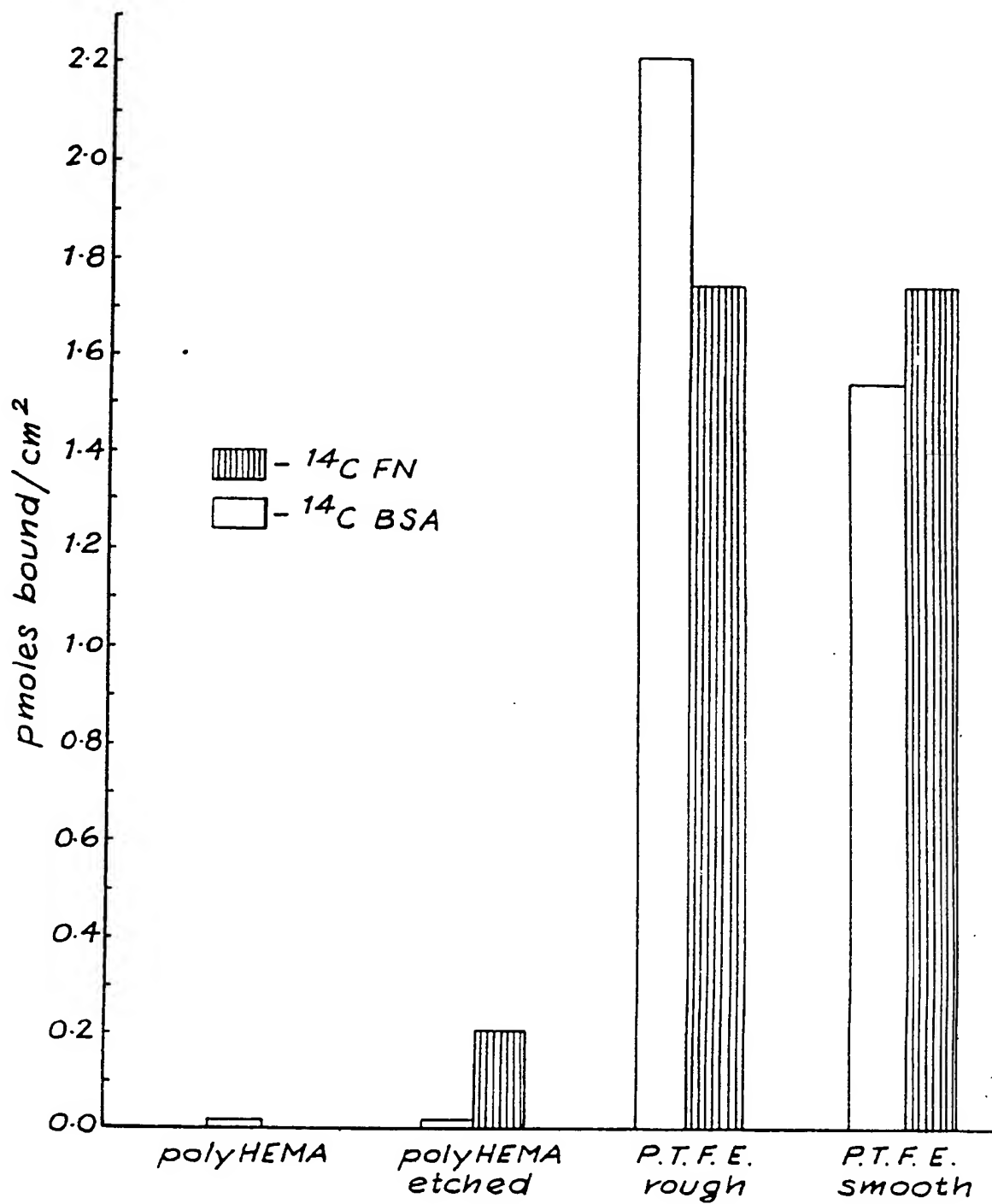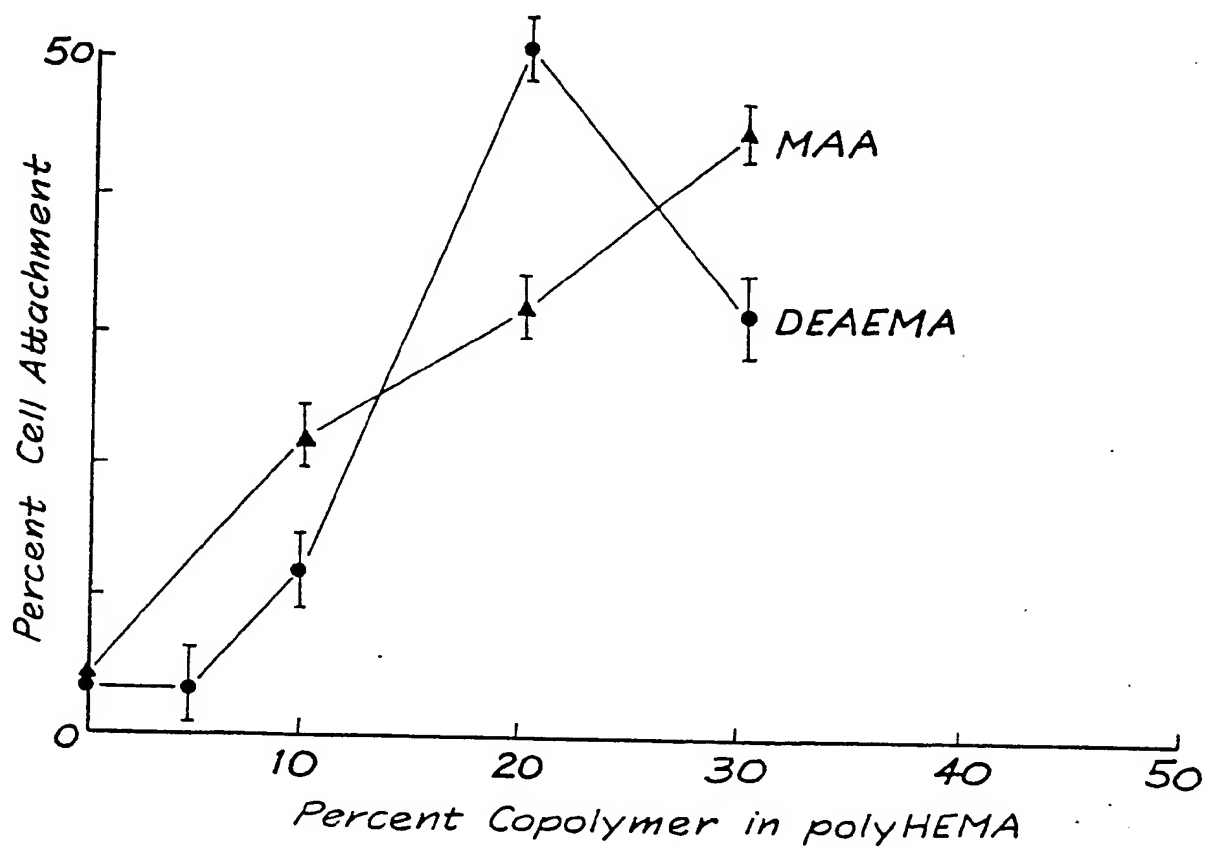
FIG. 1

FIG. 2

FIG. 3

FIG. 4

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.^{4} C08J 7/14, 7/12

II. FIELDS SEARCHED

Minimum Documentation Searched [7]	
Classification System	Classification Symbols
IPC	C08J 7/12, 7/14, 7/16, 7/18

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [8]

AU: IPC as above;   Australian Classification 47.75712

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	GB,B, 1401233 (CESKOSLOVENSKA AKADEMIE VED) 23 July 1975 (23.07.75) See page 1 lines 20-40 page 2 lines 9-20	(1-2,4-9, 13-22)
X,Y	AU,B, 82681/82 (550604) (TOYO CONTACT LENS CO., LTD) 4 November 1982 (04.11.82) See page 5 lines 5-20	(1,2,4,6-8, 13-22)
Y	AU,A, 58860/80 (KABUSHIKI KAISHA HOYA LENS) 25 June 1981 (25.06.81)	(1-22)
A	GB,B, 1280349 (MINNESOTA MINING AND MANUFACTURING COMPANY) 5 July 1972 (05.07.72) See page 1 lines 20-25, page 2 lines 28-35	(1-22)
A	AU,B, 70019/81 (543031) (POLYPLASTICS CO., LTD) 5 November 1981 (05.11.81)	(1-22)

* Special categories of cited documents: [10]
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 May 1987 (28.05.87)	(09-06-87) 9 JUNE 1987
International Searching Authority	Signature of Authorized Officer
Australian Patent Office	/R. SAWYER

Form PCT/ISA/210 (second sheet) (January 1985)

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members					
GB 1401233	AU	47491/72	BE	78839	DE	2247739
	ES	407412	FR	2155629	IL	40498
	IT	974644	JP	48046668	NL	7213522
AU 82681/82	CA	1182614	DE	3215918	FR	2505055
	GB	2097952	JP	57181524	AU	79163/82
	CA	1187645	DE	3200479	FR	2497967
	GB	2091750	JP	58065411	US	4451629
	JP	57116318				
AU 58860/80	CA	1131106	EP	31633	JP	56088108
	PH	15569				
GB 1280349	CA	924470	DE	1937618	FR	2017948
	US	3538521				
AU 70019/81	BR	8108573	EP	39248	GB	2074586
	HK	855/84	JP	56152845	US	4418162
	WO	8103178				

END OF ANNEX